REMARKS

Claims 22-27 are pending in this application. In the Office Action mailed February 11, 2003, the Examiner rejected claims 22-27 under 35 U.S.C. §112 first paragraph for lack of enablement and second paragraph as being indefinite.

Applicants respectfully request that the Examiner consider the following remarks in response to the Office Action.

I. Rejection of Claims Under 35 U.S.C. §112, First Paragraph- Enablement

The Examiner rejected claims 22-27 as being drawn to subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

Applicants respectfully traverse this rejection and request that the Examiner reconsider this rejection. The pending claims are directed to antibodies against proteins encoded by the polypeptide shown in Figure 32 (SEQ ID NO: 83, PRO361). The specification, on pages 81 to 89, teaches one skilled in the art several methods to make antibodies directed against PRO361. For example, on page 81, the specification teaches one skilled in the art how to make polyclonal anti-PRO antibodies. Polyclonal antibodies can be raised in a mammal, typically by injecting the immunizing agent and/or adjuvant into the mammal with multiple subcutaneous or intraperitoneal injections. The immunizing agent may include the PRO polypeptide or a fusion protein thereof. It may be useful to conjugate the immunizing agent to a protein known to be immunogenic in the mammal being immunized. The immunization protocol may be selected by one skilled in the art without undue experimentation.

The specification on page 89, lines 8-9, also discloses that anti-PRO antibodies may be used in diagnostic assays for PRO, e.g., detecting its expression in specific cells, tissues, or serum. One skilled in the art knows how to use antibodies for detecting expression of a given polypeptide in tissue

samples, for example. Applicants acknowledge that detection conditions may be varied depending on the tissues to be screened and the expression levels of the specific protein in the specific tissue to be screened, however variations in the detection conditions clearly cannot be considered undue experimentation. According to the MPEP § 2164.01, "the fact that experimentation may be complex does not necessarily make it undue, if the art typically engages in such experimentation." Thus, one skilled in the art would be enabled by the specification to make PRO361 antibodies and to use the PRO361 antibodies to detect expression of PRO361 in tissues. *See Int. J. Cancer* 43: 1072-1076, 1989, as an example.

In *In re Wands,* 858 F. 2d 731, 737 (Fed. Cir. 1988), the Federal circuit stated:

> The determination of what constitutes undue experimentation in a given case requires the application of a standard of reasonableness, having due regard for the nature of the invention and the state of the art (citations omitted). The text is not merely quantitative, since *a considerable amount of experimentation is permissible*, if it is merely routine, or *if the specification provides a reasonable amount of guidance* with respect to the direction in which the experimentation should proceed (emphasis added).

The *Wands* court set forth the following factors to analyze in determining whether an application is enabled: (1) the nature of the invention, (2) the state of the prior art, (3) the relative skill of those in the art, (4) the level of predictability in the art, (5) the existence of working examples, (6) the breadth of the claims, (7) the amount of direction or guidance by the inventor, and (8) the quantity of experimentation needed to make or use the invention. *In re Wands*, 858 F.2d 731, 737 (Fed. Cir. 1988).

Applicants respectfully assert that the deposit of Clone DNA45410-1250 with the American Type Culture Collection (ATCC), combined with the methods for making and using the claimed invention described in the specification and the

methods well known to those of skill in the art enable a skilled artisan to practice the full scope of the claimed invention.

Enablement of a single utility is sufficient. *In re Vaeck*, 947 F.2d 488, 496 (Fed. Cir. 1991).

Applicants respectfully assert that the claimed invention is fully enabled. Accordingly, Applicants request that the Examiner reconsider and withdraw the rejection under § 112, first paragraph.

II. Rejection of Claims Under 35 U.S.C. §112, Second Paragraph-Indefiniteness

The Examiner rejected claims 22 and 27 as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as the invention. The Examiner rejected claims 22 and 27, stating that it is unclear what the difference between the two claims is and what subject matter each claim is meant to encompass given that antibody binding is determined by variable regions structure and is a specific reaction. Applicants have withdrawn claim 22 and rewritten claim 27 in independent form. Claims 23-26 have been amended to depend from independent claim 27. Furthermore, Applicants respectfully assert that the term "specific binding" is a term well-known to one skilled in the art and Applicants did not believe it necessary to include an explicit definition. One skilled in the art would understand the subject matter encompassed by claim 27, claiming an antibody that specifically binds to the polypeptide shown in Figure 32 (SEQ ID NO: 83).

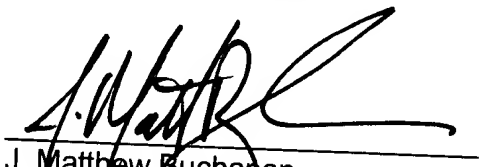
Thus, based on Applicants amendment, the Examiner's rejection is obviated. Applicants respectfully request that the Examiner withdraw the rejection of claims 23-27.

CONCLUSION

Applicants believe this Amendment and Request for Reconsideration fully responds to the Office Action. Applicants respectfully request the Examiner grant early allowance of this application. The Examiner is invited to contact the undersigned attorney for the Applicant via telephone if such communication would expedite this application.

Applicants believe no fee is due in connection with the filing of this Reply, however, should any fees be deemed necessary for any reason relating to this paper, the Commissioner is hereby authorized to deduct said fees from Brinks Hofer Gilson & Lione Deposit Account No. 23-1925. A duplicate copy of this document is enclosed.

Respectfully submitted,



J. Matthew Buchanan
Registration No. 47,459
Attorney for Applicant

BRINKS HOFER GILSON & LIONE
P.O. BOX 10395
CHICAGO, ILLINOIS 60610
(312) 321-4200

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A CORE PROTEIN EPITOPE OF THE POLYMORPHIC EPITHELIAL MUCIN DETECTED BY THE MONOCLONAL ANTIBODY SM-3 IS SELECTIVELY EXPOSED IN A RANGE OF PRIMARY CARCINOMAS

Anne GIRLING[1], Jirina BARTKOVA[3,4], Joy BURCHELL[2], Sandra GENDLER[2], Cheryl GILLETT[1] and Joyce TAYLOR-PAPADIMITRIOU[2]

[1]*Imperial Cancer Research Fund Clinical Oncology Unit; Guy's Hospital, London SE1 9RT;* [2]*Imperial Cancer Research Fund Laboratories, P.O. Box 123, Lincoln's Inn Fields, London WC2A 3PX, UK; and* [3]*Research Institute of Clinical and Experimental Oncology, Zluty Kopec 7, 656 01 Brno, Czechoslovakia.*

The monoclonal antibody (MAb) SM-3, which was raised to chemically deglycosylated milk mucin, reacts with an epitope present on the core protein of this mucin which we have referred to as PEM (polymorphic epithelial mucin). Although this mucin is abundantly expressed by both the lactating breast and breast carcinomas, the antibody SM-3 shows very little or no reactivity on the former but does react with 92% of breast carcinomas. Furthermore, SM-3 stains primary carcinomas of the lung, colon and ovary, but on the corresponding normal tissue the epitope is expressed at a much reduced level or not at all. These results indicate that an epitope masked in the normal mucin is exposed in the mucin produced by tumour cells, perhaps due to aberrant glycosylation. An extensive immunohistochemical study of other normal tissues reveals that the majority show only weak focal staining with SM-3 or none at all, the distal tubules and collecting ducts of the kidney, and sebaceous glands being the only normal tissues studied to show homogeneously positive staining.

Among the large number of MAbs in routine diagnostic use are those directed against antigens on the membranes of epithelial cells, many of which are now known to recognize different epitopes on high-molecular-weight glycoprotein molecules or mucins. As mucins are produced by both normal epithelial cells and cancers of epithelial origin from a variety of anatomical sites, MAbs to them can show reactivity with more than one tissue or tumour type. Many MAbs including HMFG-1 and HMFG-2 (Taylor-Papadimitriou *et al.*, 1981; Burchell *et al.*, 1983) recognize different, tumour-associated epitopes on a high-molecular-weight (>400 kDa) glycoprotein molecule found in human milk (Burchell and Taylor-Papadimitriou, 1989). This milk mucin has been termed PAS-0 by Shimizu and Yamauchi (1982), who first purified it, EMA (epithelial membrane antigen) by Heyderman *et al.* (1979), MAM-6 by Hilkens *et al.* (1984) and NPG (non-penetrating glycoprotein) by Ceriani *et al.* (1983). Because the mucin exhibits a high degree of polymorphism at both the protein and DNA levels (Swallow *et al.*, 1987; Gendler *et al.*, 1987) we refer to this mucin as PEM [polymorphic epithelial mucin (Taylor-Papadimitriou and Gendler, 1988)].

Immunohistochemical studies with the HMFG-1 and HMFG-2 MAbs have shown that, while they react with many mammary carcinomas, they also show strong reactivity with secretory breast tissue and benign tumours and, to a lesser degree, with resting breast and some other epithelial tissues (Arklie *et al.*, 1981; Taylor-Papadimitriou *et al.*, 1986). Other MAbs to different epitopes on the PEM mucin show similar patterns of reactivity (Foster *et al.*, 1982; Price *et al.*, 1985; Burchell and Taylor-Papadimitriou, 1989). We have recently described the production of MAbs to the chemically deglycosylated core protein of the polymorphic epithelial mucin (Burchell *et al.*, 1987), and preliminary immunohistochemical studies with one of these antibodies, SM-3, gave particularly interesting results. In marked contrast to HMFG-1 and HMFG-2, virtually no reactivity was seen in normal secretory or resting breast tissue, although 92% of primary mammary carcinomas showed positive staining with the antibody. We have now extended these preliminary observations to see whether a similar differential expression of the SM-3 epitope is seen in other epithelial tissues and tumours and how extensively the determinant is expressed on normal tissues. Here we report the results of an immunohistochemical study on staining with SM-3 of primary carcinomas of the colon, lung and ovary as well as of normal tissue from these and other sites. We also report the results of further work on primary breast carcinomas and a large number of normal and secretory breast biopsies.

MATERIAL AND METHODS

Selection of tissues

Most normal tissue was obtained at autopsy, but in some instances it was selected from surgical specimens. Autopsy tissue was obtained within hours of death to minimize autolysis. However, if any signs of autolysis were seen in preliminary examination of sections stained with haematoxylin and eosin, these specimens were not used. Obtaining well-preserved autopsy specimens from the gastro-intestinal tract and the lung was particularly difficult and these were therefore obtained as surgical specimens. In some of these latter a tumour was present and in these cases, the tissue selected was from a site as far away from the tumour as possible. Carcinoma tissue was obtained from specimens sent for routine diagnostic purposes to the Histopathology Laboratories at the following London hospitals: Guy's, The Brompton, University College and St. Mark's.

Antibody

The development and characterization of the SM-3 MAb has been described by Burchell *et al.* (1987). Briefly, it was raised against deglycosylated mucin (purified by immunoaffinity chromatography from human skimmed milk) and was selected for its reactivity with partially and totally stripped mucin and lack of reactivity with intact mucin. It is an MAb of the IgG1 class and expresses a λ light chain (Burchell, pers. commun.).

Staining method

A conventional indirect immunoperoxidase technique was used as described by Bartek *et al.* (1985). Initially, tissue was fixed in a variety of different fixatives, so that the effect of different methods of fixation could be assessed and compared to staining of unfixed frozen tissue. These studies showed that methacarn (methanol:chloroform:acetic acid 60:30:10) was the most satisfactory fixative for use with SM-3. Tissues and tumours were therefore fixed in methacarn, processed routinely, embedded in paraffin wax and cut in 3- to 4-μm sections for staining.

[4]Present address: ICRF Research Fund Laboratories, P.O. Box 123, Lincoln's Inn Fields, London WC2A 3PX, UK.

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In all cases, the results of staining with SM-3 were compared with those seen with HMFG-2, staining being performed in parallel. A positive control (a primary breast carcinoma showing a uniform pattern of staining with SM-3) was included with each batch of slides to ensure reproducibility of results from one occasion to another. Negative controls (in which PBS buffer replaced the primary antibody) were included for each case.

RESULTS

Fixation of tissues and tumours

No differences in the staining patterns obtained with SM-3 were observed when methacarn-fixed and frozen tissues or tumours were used. In several tumours and tissues, the effect of 3 different formalin fixatives was examined. Acid formalin containing 40% methanol (methanol: 40% formalin:acetic acid:water 8:2:1:9) was as good as methacarn in preserving the SM-3 determinant, but use of neutral buffered formalin (4% formaldehyde in 0.15 M NaCl) or acid formalin (40% formaldehyde:acetic acid:distilled water 2:1:17) resulted in a reduction in the degree of staining with SM-3. Assessment of other fixatives showed that Carnoy's and Bouin's gave comparable results to methacarn fixation. In the studies reported here, all tissues and tumours were fixed in methacarn.

Staining of breast tissues and tumours

A pilot study suggested that SM-3 was highly selective in showing positive staining of primary and metastatic breast cancers, while staining of normal breast tissue was weak or undetectable. We have extended these initial observations to include 122 primary carcinomas, 17 normal resting breast biopsies and 13 secretory breast biopsies.

Table I shows the results of staining the 122 primary carcinomas with SM-3. In keeping with previously published results (Burchell *et al.*, 1987) the overall incidence of SM-3 positivity was 92%. Tumours of different histological types and grades were examined but no difference in staining pattern was seen according to either feature. In many of the tumours the staining pattern was strong but heterogeneous. In some cases staining was concentrated on the cell membranes, although cytoplasmic staining was present to a greater or lesser extent in all cases (Fig. 1*a*). In tumours which showed glandular differentiation, the strongest staining was seen at the surface of cells lining the glandular lumina.

When compared with HMFG-2, which stained 121 of the 122 tumours, staining with SM-3 was weaker and more heterogeneous in 103 cases (84%), a similar pattern being seen in 19 cases. The staining with HMFG-2, although usually more intense, was similar in distribution to that described for SM-3. One tumour (a metaplastic carcinoma) failed to stain with either antibody.

TABLE I – PRIMARY CARCINOMAS OF THE BREAST: RESULTS OF IMMUNOHISTOCHEMICAL STAINING

Histological type	Total number	Number SM-3-positive	Number HMFG-2-positive
Infiltrating ductal			
Grade I	7	6	7
Grade II	41	37	41
Grade III	29	29	29
Infiltrating lobular	29	28	29
In situ carcinoma			
Ductal	3	3	3
Lobular	1	1	1
Special types			
Mucoid	3	1	3
Medullary	4	4	4
Tubular	2	2	2
Adenoid cystic	2	1	2
Metaplastic carcinoma	1	0	1
Total	122	112 (92%)	

Examination of normal breast tissue showed that, of 17 normal resting breast biopsies, 9 showed no staining with SM-3 and in the remaining 8 the staining observed was weak and focal. Of 13 biopsies showing secretory changes, 5 were SM-3 negative. In the remaining 8, any positivity observed was extremely weak and focal, being confined to only very small areas of the section. This pattern is in marked contrast to that seen with HMFG-2 which stains secretory tissue strongly and homogeneously, and stains 50–60% of lobules in the resting breast.

As we have previously reported, most benign breast lesions showed either no reaction or weak and focal staining with SM-3, the strongest staining pattern being seen in epithelium showing apocrine metaplasia (Burchell *et al.*, 1987).

Staining of colonic tissues and tumours

Seventeen primary tumours of the colon and rectum were studied. All were moderately differentiated adenocarcinomas and all showed some positivity with SM-3. The pattern and intensity of staining, however, showed some variation. In 6 tumours the staining was homogeneous, strong and similar to that seen with HMFG-2. In the other 11, staining with SM-3 was heterogeneous and less intense than with HMFG-2. In these cases the strongest staining with both MAbs was noted at the luminal edges of cells lining glandular structures, whereas in the uniformly staining cases the intensity of staining in the cytoplasm and at the luminal margins was similar (Fig. 1*b*).

Although staining with SM-3 of the normal mucus-secreting epithelium of the colon could be detected, this was consistently extremely weak, in marked contrast to the strong staining seen in the colonic carcinomas (Table III).

Staining of lung tissues and tumours

In the 15 primary lung tumours studied, the pattern and intensity of staining showed some variation with histological type. The strongest reactivity was seen in adenocarcinomas (Fig. 1*c*). Tumours of neuroendocrine origin either failed to stain or showed weak positivity in a few cells only, and 2 undifferentiated large-cell carcinomas showed a similar pattern, while an intermediate level of staining was seen in squamous carcinomas. The staining patterns seen in the lung tumours are summarized in Table II.

When the results with SM-3 and HMFG-2 were compared, the latter stained a greater proportion of the total number of tumours and in general the staining was stronger with HMFG-2. In many cases, however, the staining pattern with both antibodies was heterogeneous and the intensity of staining varied between different areas of the same tumour.

Examination of 5 samples of normal lung tissue showed very weak staining of the bronchial epithelium, and heterogeneous staining of pneumocytes in the alveoli. The mesothelium lining the pleura was consistently negative (see Table III).

Staining of ovarian tissues and tumours

All 5 primary carcinomas of the ovary which were examined stained positively with SM-3 (Fig. 1*d*). Two of the tumours were poorly differentiated adenocarcinomas and, in these, staining with SM-3 was similar to staining with HMFG-2. In 1 case the pattern was heterogeneous but in the other more homogeneous. Of the other 3 tumours, one (a mesonephroid carcinoma) showed a similar staining pattern with the 2 MAbs but in the others (1 mucinous and 1 serous adenocarcinoma), stain-

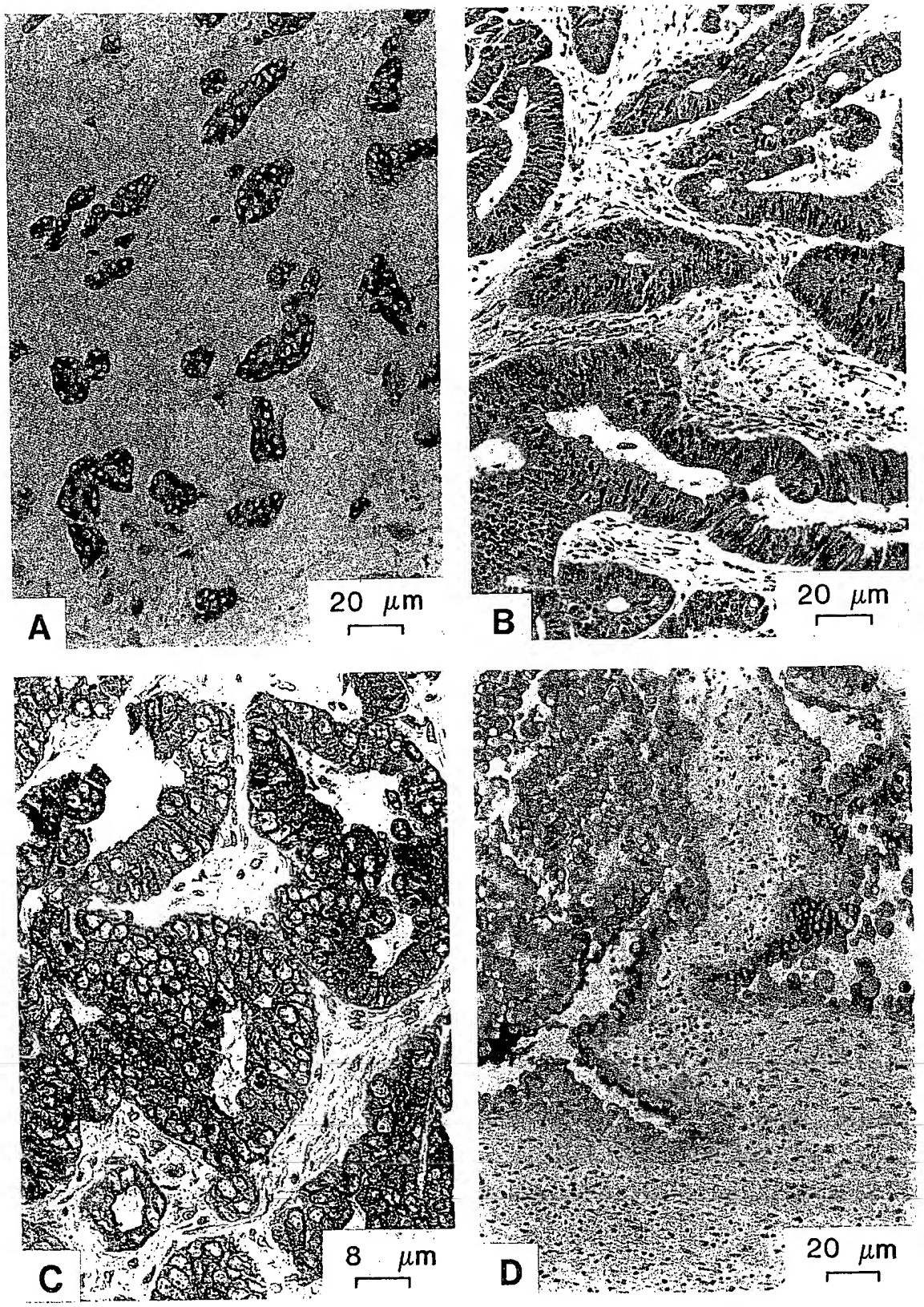

FIGURE 1 – Immunoperoxidase staining with SM-3 of primary carcinomas. *(a)* Infiltrating ductal carcinoma of breast; *(b)* adenocarcinoma of colon; *(c)* adenocarcinoma of lung; *(d)* mesonephroid carcinoma of ovary.

TABLE II – PRIMARY CARCINOMAS OF COLON, LUNG AND OVARY: RESULTS OF IMMUNOHISTOCHEMICAL STAINING

Tissue and tumor type (Total number of cases)	Number of cases	SM-3-positive	HMFG-2-positive
Colon and rectum (17)		17	17
Adenocarcinoma	17	17	17
Lung (15)		10	13
Squamous carcinoma	4	3	4
Adenocarcinoma	5	5	5
Carcinoid tumours	3	1 (weak)	2 (weak)
Large-cell undifferentiated	2	1	2
Spindle-cell	1	0	0
Ovary (5)		5	5
Adenocarcinoma	2	2	2
poorly differentiated mucinous carcinoma	1	1	1
serous carcinoma	1	1	1
mesonephroid carcinoma	1	1	1

ing with SM-3 was more heterogeneous and less intense than with HMFG-2.

The surface epithelium of normal ovarian tissues taken from 15 different individuals showed no positive staining with SM-3.

Staining of other normal tissues

The results of staining normal human tissues from a variety of sites with the antibody SM-3 are summarized in Table III. Reactivity was seen only within epithelial structures and was never observed in tissues of either mesenchymal or lymphoid origin. The only tissues showing a homogeneously positive pattern of staining were the distal tubules and collecting ducts of the kidney and the sebaceous gland. Any other positive reaction was generally focal and often weak.

DISCUSSION

The immunohistochemical study described here confirms the earlier observation that the SM-3 reactive epitope on the core protein of human polymorphic epithelial mucin is selectively unmasked in breast cancers, and only marginally detectable on the mucin produced by the normal gland. The SM-3 antibody was raised against the deglycosylated mucin which was first purified in its native form from milk by immunoaffinity chromatography using the HMFG-1 antibody. Since the SM-3 antibody was selected for lack of reaction with the fully glycosylated mucin, it is not surprising that it shows a negative reaction with the pregnant or lactating gland which produces this mucin. The positive reaction of SM-3 with breast cancers suggests that the mucin is aberrantly glycosylated in malignant cells, resulting in the unmasking of the SM-3 epitope.

Support for this idea comes from recent studies analyzing the structure of the oligosaccharide side-chains of the mucin purified from human milk (Hanisch *et al.*, 1989) and of the mucin produced by a breast cancer cell line, BT20 (Hull *et al.*, 1988); the normal mucin contains extended poly-N-acetyl-lactosamine side chains (made up of 4–14 sugars), while the BT20 mucin has short chains made up of only 3–4 sugars. Clearly, the longer chains might be expected to mask larger stretches of the core protein than would be masked by the short chains, and core protein epitopes would consequently be selectively exposed in the cancer-associated mucin.

The selective reactivity of the SM-3 antibody with breast cancers has identified a difference between the normal and malignant mammary epithelial cell in mucin processing. It was of interest, therefore, to see whether a similar difference is found between other normal and malignant epithelial cells,

TABLE III – NORMAL TISSUES: RESULTS OF IMMUNOHISTOCHEMICAL STAINING WITH SM-3

Tissue (Number of cases studied)	Pattern of staining[3]		
	Uniformly positive	Weak and/or heterogeneous	Uniformly negative
Breast			
Resting (9)			–
Resting (8)		±	
Secretory (5)			–
Secretory (8)		–/+	
Lung (5)			
Bronchial mucosa		–/+	
Alveoli (type 1 and 2 pneumocytes)		+/–	
Pleura			–
Colon (10)[1]		–/+	
Ovary (15)			–
Rete ovary (1)		+/–	
Fallopian tube		+/–	
Skin			
Interfollicular epidermis (6)			–
Sebaceous gland (6)	+		
Hair follicle (3)			–
Sweat gland (eccrine) (3)		+/–	
Endometrium (5)		±	
Endocervix (4)		±	
Ectocervix (4)			–
Vagina (1)			–
Vulva (1)			–
Placenta (1)			–
Prostate (3)		+/–	
Testis (2)			–
Epididymis (1)		–/+	
Spermatic cord (2)		+/–	
Kidney (3)			
Glomeruli			–
Proximal tubules			–
Distal tubules	+		
Collecting ducts	+		
Urinary bladder (1)			–
Ureter (1)		±	
Thymus (2)			–
Adrenal (1)			–
Thyroid (2)			–
Pancreas (3)			
Acini		±	
Ducts			–
Islets of Langerhans			–
Liver (3)			–
Gall bladder (3)		+/–	
Tongue (2)			–
Oesophagus (2)			–
Stomach[2] (3)			
Surface epithelium		–/+	
Glands		+/–	
Small intestine (1)			–
Appendix (1)			–
Salivary gland			
Parotid (5)		+/–	
Submandibular (2)		+/–	
Sublingual (4)			–
Larynx (1)			–

[1]Tumour-associated tissue in 2 cases.–[2]Tumour-associated tissue in 1 case.–[3]+ Uniformly positive; – uniformly negative; –/+ very weak, barely detectable; ± generally very weak and focal; +/– stronger foci, but very heterogeneous in distribution.

particularly those associated with the common carcinomas of colon, lung and ovary. It is difficult to assess the level of production of the PEM mucin by the normal cells in these organs using MAbs, since there may be tissue-specific differences in mucin processing leading to differences in the profile of antigenic determinants expressed. However, our results show definitively that the SM-3 core protein epitope of the mucin is found abundantly on primary carcinomas of the lung, colon and ovary, as well as on breast carcinomas, but on the corresponding normal tissues it is expressed at a much reduced level (in breast, lung and colon) or not at all (in ovary). This observation suggests that the mucin is produced by carcinomas other than those of the breast, and furthermore is underglycosylated so that the SM-3 epitope is exposed.

Practical applications of an antibody showing the kind of selectivity of reaction shown by SM-3 include the *in vivo* imaging of tumours (Granowska *et al.*, 1984) and in antibody-guided therapy (Epenetos *et al.*, 1984). We therefore examined as many other normal human tissues as possible to ascertain the extent of reactivity of the antibody. The results show surprisingly little cross-reactivity with normal tissues, sebaceous glands and kidney tubules and collecting ducts being the only homogeneously positively staining tissues. Some other epithelial tissues showed weak and/or focal staining, and all non-epithelial tissues were negative.

While the selectivity of SM-3 in reacting with carcinomas is impressive, its reaction with many tumours is not as strong as that shown by the antibody HMFG-2. HMFG-2 also reacts with a core protein epitope of the mucin, but a certain number of these determinants are exposed in the fully glycosylated molecule (Burchell *et al.*, 1983). We have recently obtained partial cDNA clones coding for the core protein of the mucin gene, and have identified the immunogenic region containing the SM-3 and HMFG-2 epitopes as being made up of tandem repeats of 20 amino acids (Gendler *et al.*, 1987). Both antibodies show strong reactivity with a synthetic peptide corresponding to the tandem repeat (Gendler *et al.*, 1988) and it is now possible to map in detail the epitopes recognized by SM-3 and HMFG-2. This being so, it should be possible to develop new antibodies with a similar specificity to SM-3 but with a higher affinity.

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REFERENCES

ARKLIE, J., TAYLOR-PAPADIMITRIOU, J., BODMER, W., EGAN, M. and MILLIS, R., Differentiation antigens expressed by epithelial cells in the lactating breast are also detectable in breast cancers. *Int. J. Cancer*, **28**, 23–29 (1981).

BARTEK, J., DURBAN, E.M., HALLOWES, R.C. and TAYLOR-PAPADIMITRIOU, J., A subclass of luminal epithelial cells in the human mammary gland, defined by antibodies to cytokeratins. *J. Cell Sci.*, **75**, 17–33 (1985).

BURCHELL, J., DURBIN, H. and TAYLOR-PAPADIMITRIOU, J., Complexity of expression of antigenic determinants, recognized by monoclonal antibodies HMFG-1 and HMFG-2, in normal and malignant human mammary epithelial cells. *J. Immunol.*, **131**, 508–513 (1983).

BURCHELL, J., GENDLER, S., TAYLOR-PAPADIMITRIOU, J., GIRLING, A., LEWIS, A., MILLIS, R. and LAMPORT, D., Development and characterization of breast cancer reactive monoclonal antibodies directed to the core protein of the human milk mucin. *Cancer Res.*, **47**, 5476–5482 (1987).

BURCHELL, J. and TAYLOR-PAPADIMITRIOU, J., Antibodies to human milk fat globule molecules. *Cancer Invest.*, **17**, 53–61 (1989).

CERIANI, R.L., PETERSON, J.A., LEE, J.Y., MONCADA, R. and BLANK, E.W., Characterisation of cell surface antigens of human mammary epithelial cells with monoclonal antibodies prepared against human milk fat globule. *Somatic Cell Genet.*, **9**, 415–427 (1983).

EPENETOS, A., COURTENAY-LUCK, N., HALNAN, K., HOOKER, G., HUGHES, J., KRAASZ, T., LAMBERT, J., LAVENDER, J., MACGREGOR, W., MCKENZIE, C., MUNRO, A., MYERS, M., ORR, J., PEARSE, E., SNOOK, D., WEBB, B., BURCHELL, J., DURBIN, H., KEMSHEAD, J. and TAYLOR-PAPADIMITRIOU, J., Antibody-guided irradiation of malignant lesions: three cases illustrating a new method of treatment. *Lancet*, **II**, 1441–1443 (1984).

FOSTER, C., EDWARDS, P.A., DINSDALE, E.A., *et al.*, Monoclonal antibodies to the human mammary gland. I. Distribution of determinants in non-neoplastic mammary and extramammary tissues. *Virchows Arch. (Path. Anat.)*, **394**, 279–293 (1982).

GENDLER, S.J., BURCHELL, J.M., DUHIG, T., LAMPORT, D., WHITE, R., PARKER, M. and TAYLOR-PAPADIMITRIOU, J., Cloning of partial cDNA encoding differentiation and tumor-associated mucin glycoproteins expressed by human mammary epithelium. *Proc. nat. Acad. Sci. (Wash.)*, **84**, 6060–6064 (1987).

GENDLER, S., TAYLOR-PAPADIMITRIOU, J., DUHIG, T., ROTHBARD, J. and BURCHELL, J., A highly immunogenic region of a human polymorphic epithelial mucin expressed by carcinomas is made up of tandem repeats. *J. biol. Chem.*, **263**, 12820–12823 (1988).

GRANOWSKA, M., SHEPHERD, J., BRITTON, K.E., WARD, B., MATHER, S., TAYLOR-PAPADIMITRIOU, J., EPENETOS, A.A., CARROLL, M.J., NIMMON, C.C., HAWKINS, L.A., SLEVIN, M., FLATMAN, W., HORNE, T., BURCHELL, J., DURBIN, H. and BODMER, W., Ovarian cancer: diagnosis using ^{123}I-monoclonal antibody in comparison with surgical findings. *Nucl. Med. Commun.*, **5**, 485–499 (1984).

HANISCH, F.-G., UHLENBRUCK, G., PETER-KATALINIC, J., EGGE, H., DABROWSKI, J. and DABROWSKI, U., Structures of neutral O-linked polylactosaminoglycans on human skim milk mucins. A novel type of linearly extended poly-N-acetyl-lactosamine backbones with Galβ(1–4)GlcNAc-β(1–6) repeating units. *J. biol. Chem.*, **264**, 872–883 (1989).

HEYDERMAN, E., STEELE, K. and ORMEROD, M.G., A new antigen on the epithelial membrane: its immunoperoxidase localization in normal and neoplastic tissues. *J. clin. Pathol.*, **32**, 35–39 (1979).

HILKENS, J., BUIJS, F., HILGERS, J., HAGEMAN, P., CALAFAT, J., SONNENBERG, A. and VAN DER VALK, M., Monoclonal antibodies against human milk fat globule membranes detecting differentiation antigens of the mammary gland and its tumours. *Int. J. Cancer*, **34**, 197–206 (1984).

HULL, S.R., BRIGHT, A., CARRAWAY, K.L., ABE, M. and KUFE, D., Oligosaccharides of the DF3 antigen of the BT-20 human breast carcinoma cell line. *J. Cell. Biochem.*, Suppl. **12E**, 130 (1988).

PRICE, M., EDWARDS, S., OWAINATI, A., BULLOCK, J.E., FERRY, B., ROBINS, R.A. and BALDWIN, R.W., Multiple epitopes on a human breast carcinoma associated antigen. *Int. J. Cancer*, **36**, 567–572 (1985).

SHIMIZU, M. and YAMAUCHI, K., Isolation and characterisation of mucin-like glycoproteins in human milk fat globule membranes. *J. Biochem.*, **91**, 515–524 (1982).

SWALLOW, D.M., GENDLER, S., GRIFFITHS, B., CORNEY, G., TAYLOR-PAPADIMITRIOU, J. and BRAMWELL, M.E., The human tumour-associated epithelial mucins are coded by an expressed hypervariable gene locus PUM. *Nature (Lond.)*, **328**, 82–84 (1987).

TAYLOR-PAPADIMITRIOU, J. and GENDLER, S.J., Molecular aspects of mucins. *Cancer Rev.* (1989) (In press).

TAYLOR-PAPADIMITRIOU, J., MILLIS, R., BURCHELL, J., NASH, R., PANG, L. and GILBERT, J., Patterns of reaction of monoclonal antibodies HMFG-1 and 2 with benign breast tissues and breast carcinomas. *J. exp. Path.*, **2**, 247–260 (1986).

TAYLOR-PAPADIMITRIOU, J., PETERSON, J.A., ARKLIE, J., BURCHELL, J., CERIANI, R.L. and BODMER, W.F., Monoclonal antibodies to epithelium-specific components of the human milk fat globule membrane: production and reaction with cells in culture. *Int. J. Cancer*, **28**, 17–21 (1981).